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ARS CFDA NO. 10.001		Budget Object Class Code: 4117	
UNITED STATES DEPARTMENT OF AGRICULTURE EXTRAMURAL AGREEMENT		TYPE OF RESEARCH AGREEMENT Grant Agreement	
TITLE OF PROJECT Plant Exploration in Northern Chile to Collect Wild Tomato Species with Emphasis on <i>Solanum lycopersicoides</i> and <i>S. siliens</i>		AGREEMENT NO. 59-1275-1-015	TYPE OF ACTION Amendment No. 01
		PERIOD OF AGREEMENT 15-Feb-01 thru 15-Feb-02	
		FEDERAL OBLIGATION \$14,580.00	CHANGE IN FEDERAL OBLIGATION <input type="checkbox"/> <input type="checkbox"/> \$3,000.00
AGENCY (Name and Address) USDA, ARS, BA, FMOD Procurement and Contracting Section Bldg. 003, Rm. 329, BARC-West 10300 Baltimore Ave Beltsville, Maryland 20705-2350		CRIS NO. 1275-21000-141-07G	AUTHORITY 7 U.S.C. 3318 (a)
ARS AUTHORIZED DEPARTMENTAL OFFICER'S DESIGNATED REPRESENTATIVE (Name and Address) Dr. Edward J. Garvey USDA, ARA, BA, PSI, NGRIL Bldg. 003, Rm. 409, BARC-West 10300 Baltimore Ave. Beltsville, Maryland 20705-2350		OBLIGATION DISTRIBUTION Accounting Code 1011275115 Amount \$3,000.00	
ARS FINANCE OFFICE (Complete Mailing Address) USDA, National Finance Center ARB, RS No. 4, Tano Bldg. P.O. Box 53326 New Orleans, Louisiana 70153		RECIPIENT (Name and Address) Regents, University of California Office of the Vice Chancellor for Research Sponsored Programs, 118 Everson Hall University of California One Shields Avenue Davis, California 95616	
		RECIPIENT'S DESIGNATED REPRESENTATIVE (Name and Address) Dr. T. Roger Chetelat University of California-Davis Department of Vegetable Crops Tomato Genetics Resource Center Davis, California 95616 Email: trchetelat@ucdavis.edu Phone: 530-752-6726 Fax: 530-752-9659	

APPLICABLE PROVISIONS AND REGULATIONS

This Agreement includes the following:

- ☐ Statement of Work; or
- ☐ Project Summary; or
- ☐ Proposal; and
- ☐ Budget (Form ARS-454/455)
- ☒ USDA Civil Rights Poster (Form AD-475A); and

Provisions:

- ☐ General (Form ARS-452)
- ☐ General (Form REE-22)
- ☐ Special (Form ARS-453)

Regulations:

- ☐ 7 CFR 3015.205 (by reference)
- ☐ 7 CFR 3016 (by reference)
- ☐ 7 CFR 3019 (by reference)
- ☐ 7 CFR 3015.175 (b), Copyrights (by reference)
- ☐ 37 CFR Part 401.14, Patents and Inventions (by reference)

Appendices and Schedules:

- ☐ Appendix A - Application for Funding (ARS-403)
- ☐ Appendix B - Current and Pending Federal Financial Assistance Support (ARS-408)
- ☐ Appendix C - Research Assurance Statement (ARS-411)
- ☐ Appendix D - Civil Rights Assurance Certification (ARS-405)
- ☐ Appendix E - Civil Rights Questionnaire (ARS-406)
- ☐ Appendix F - Certification Regarding Debarment, Suspension and other Responsibility Matters - Primary Covered Transactions (AD-1047)
- ☐ Appendix G - Certification Regarding Debarment, Suspension and other Responsibility Matters - Lower Tier Covered Transactions (AD-1048)
- ☐ Appendix H - Certification Regarding Drug-Free Workplace Requirements - Non-Individuals (AD-1049)
- ☐ Appendix I - Certification Regarding Drug-Free Workplace Requirements - Individuals (AD-1050)
- ☐ Appendix J - Certification/Disclosure Requirements Related to Lobbying (SF-LLL)
- ☐ Appendix K - Intellectual Property Rights (Applicable to International Agreements only)
- ☐ Appendix L - Report of Inventions and Subcontracts

Payment:

- ☒ HHS/Payment Management System
- ☐ Treasury Check/EFT
- ☐ Advance Payment Authorized
- ☐ Pre-Award Costs Authorized (See Below)

Reporting Requirements:

- Submit: ☐ Performance Reports ☐ Financial Reports
- ☐ Quarterly
 - ☐ Semi-annual
 - ☐ Annual
 - ☐ Final

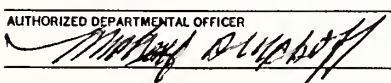
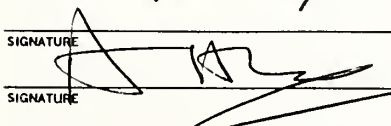
To: ☐ Annual Report of Inventions and Subcontracts

- ☐ ADODR
- ☐ Sponsoring Organization

Other (Specify):

- ☐ Form ARS-451 - page 2 (Organization Certification)
- ☐ ADODR Instructions

NOTE: This amendment is being issued to increase the Federal obligation from 11,580.00 by \$3,000.00 to \$14,580.00. All other terms and conditions remain unchanged.

FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE			
AUTHORIZED DEPARTMENTAL OFFICER 		TYPED NAME MICHAEL A. WYCKOFF	DATE 9/30/01
FOR THE PERFORMING ORGANIZATION			
(Signature of persons authorized to incur contractual obligations)			
SIGNATURE 		TYPED NAME AND TITLE Ahmad Hakim-Elahi, Director of Sponsored Programs	DATE 9/28/01
SIGNATURE		TYPED NAME AND TITLE	DATE

**Plant Exploration in Northern Chile to Collect Wild Tomato Species,
With Emphasis on *Solanum lycopersicoides* and *S. sitiens***

FINAL REPORT

Roger Chetelat
11/6/2002

Summary:

1. A total of 28 accessions were collected of *S. sitiens*, *S. lycopersicoides*, *L. chilense* and *L. peruvianum*.
2. The *S. sitiens* collections more than double the number of TGRC accessions of this species, and greatly improve *ex situ* representation of its native geographic distribution.
3. *S. lycopersicoides* was collected for the first time from the upper Rio Camarones valley, thereby improving the geographic continuity between *ex situ* accessions to the North and South.
4. Extant *S. lycopersicoides* populations are extremely limited and are threatened by grazing (some are already extinct); we therefore recommend that the authorities in Chile design and implement a conservation plan for this species.
5. As part of the benefit sharing agreement between USDA and INIA, two researchers from Chile visited UC-Davis to attend a scientific conference in September 2002.

I. Narrative:

Objectives and Rationale

The primary objective of this trip was to collect additional populations of two tomato-like nightshade species (*Solanum lycopersicoides* and *S. sitiens*) that are underrepresented in the TGRC collection. The natural range of each species is confined to limited regions of the Atacama desert in N. Chile where suitable habitat occurs. Some populations are threatened *in situ* by human influences, such as grazing, mining, and urban expansion. The number of *ex situ* collections of each species is relatively small and substantial gaps exist in the geographical distribution of the available accessions. Therefore the aim of this trip was to fill in these gaps, and thereby obtain a more comprehensive sampling of the genetic variation available in these species. A secondary objective was to collect additional populations of the wild tomatoes *Lycopersicon chilense* and *L. peruvianum*, particularly from new locations or from sites where they are sympatric with either of the *Solanum* species, and to recollect certain populations that are now extinct at the TGRC. Lastly, in addition to collecting seed, another goal of this trip was to take soil samples at plant collection sites for a comparison of the edaphic preferences of each species.

The rationale for collecting additional populations of these wild tomato species is that each possesses potentially unique phenotypic traits that are of potential interest to plant breeders, geneticists, and other researchers. The use of the wild *Lycopersicon* species (including *L. peruvianum* and *L. chilense*) as sources of beneficial traits for tomato improvement is well established; for example, resistances to over 45 diseases afflicting cultivated tomatoes have been identified in related *Lycopersicon*, and many of these resistance traits have been bred into new varieties. Utilization of the related *Solanum* species for similar purposes is impeded by the stringent crossing barriers that prevent gene flow to *L. esculentum*; however recent research in our group has demonstrated the feasibility of producing various types of genetic stocks representing the chromosomes of these nightshades in a tomato nuclear background. In this context, it is desirable to improve representation of these species in *ex situ* germplasm collections such as the TGRC.

Description of Collections

We made collections from 28 populations, including 14 of *L. chilense*, 8 of *S. sitiens*, 4 of *S. lycopersicoides* and 2 of *L. peruvianum*. The new collections of *S. sitiens* will more than double the number of accessions of this species maintained at the TGRC (from 5 to 13), and greatly increase the geographic range of populations preserved *ex situ*. For example, our collection of this species at Mina La Escondida (SAL7901) is at least 118 Km South of the any *S. sitiens* collections made prior to this trip and may represent the southern limit of its natural distribution. Despite the arid conditions of this region, we generally found large populations of *S. sitiens*, with most plants yielding seed (we collected up to 33 per site); although some plants contained only desiccated fruit, hence seed are of uncertain quality, this is nonetheless a big improvement over previous collections of this species (as few as 1 plant sampled per population). Since *S. sitiens* is an obligate outcrosser with substantial intrapopulation variation, it is essential to collect from large populations in order to establish viable *ex situ* collections and avoid problems associated with inbreeding depression.

In the case of *S. lycopersicoides*, we made several collections from new locations that help fill in a geographical gap for this species. We made the first known collections of this species in the Rio Camarones drainage. These are significant because they provide a link between populations to the North (around Putre and Palca) with the collections approx. 100 Km to the South at Camiña. In addition, we collected additional populations from the area around Camiña, but at higher elevations than previous collections. Unfortunately, we found ripe fruit on only a small proportion of plants in two out of the 4 populations we sampled (probably due to recent grazing by herbivores), hence we could not sample population diversity as well as we would have liked.

We made more collections of *L. chilense* than any of the other species, thanks to its wide distribution and large populations at most locations. Herbarium specimens were made from representative populations of each species. 15 soil samples were made at 15 collection sites to study the edaphic preferences of each; soil samples will be analyzed in the near future. Lastly, we collected samples of scat (putatively from foxes) around *S. sitiens* and *L. chilense* populations in order to identify possible seed dispersal agents.

Due to the limited quantities of seed harvested from most plants in the wild (no more than 20% of total, as per our agreement with INIA), only small backup seed samples of each accession could be deposited with INIA prior to our departure. However, we agreed that larger samples would be sent to INIA after regeneration in Davis; they would like at least 1000 seeds per population, or at least one third of the total harvest.

Regeneration of these collections by the TGRC are presently on hold while we await approval from INIA for distribution of seed samples to third parties. Under our proposed arrangement, shipments of seed would include a description (Memorandum of Understanding) of the conditions for utilization and commercialization of this germplasm. Acceptance of the germplasm would constitute acceptance of the conditions outlined in the MOU. Similar special arrangements have been made for other collections recently acquired and maintained by the U.S. National Plant Germplasm System.

Ecological Observations

S. sitiens is a xerophyte that inhabits some of the driest parts of the Atacama desert, occurring primarily within a minor range of the Andes, the Cordillera de Domeyko, at elevations of ca. 2600-3000 meters. This relatively narrow altitudinal distribution (in comparison, we found *L. chilense* from sea level to 3900 meters) is undoubtedly a reflection of its dependence on storms that make it across the Andes and produce occasional mists or light rain under specific

orographic conditions. Rainfall in this region is extremely limited. For example, the city of Calama (2,250 m) records an average of only 5.7 mm per year; last year was typical, with only 3.3 mm, however this year was relatively wet, with 16.5 mm recorded so far (a 236% increase from the average for this time of year). The increased rainfall this year, which fell mostly during the 'Bolivian winter' months of February and March, nearly forced the cancellation of our trip, as many roads were washed out, particularly in the Tarapaca region.

S. sitiens is one of the few perennial plants that thrives under the extremely xeric conditions of the Cordillera de Domeyko, and other plant taxa associated with it are primarily desert annuals. To survive during extended periods of drought, *S. sitiens* is equipped with several morphological features. Leaves are nearly glabrous (perhaps to reflect sunlight), semi-succulent, coriaceous or leathery, and fold along the veins (presumably to reduce leaf surface area). In addition, plants of this species have the ability to regenerate new shoots from the root crown following shoot death (i.e. due to prolonged drought or other causes). At most sites we collected, many plants were predominantly dead branches with a few green shoots, the latter frequently in full bloom and bearing fruit nonetheless. Fruit of *S. sitiens* desiccate shortly after ripening, without abscising from the plant, becoming a dry papery-thin shell that contains the mature seeds. On most plants we found many of these mummified fruit, a large proportion of which contained apparently healthy seeds; this allowed us to collect a larger sample of the population. In addition to finding relatively large populations of *S. sitiens*, we were also pleasantly surprised to discover this species is not particularly rare within its natural range and where suitable habitat occurs.

In contrast, *S. lycopersicoides* is much less widely distributed or abundant than the other three species we collected on this trip. One reason may be that it is limited to a very narrow range of habitats. Specifically, it prefers South facing slopes (with one exception), which are more shaded in the southern hemisphere; the only exception we observed was the population near Putre which is found at elevations up to 3,800 m where it is found on North facing slopes, presumably because low temperatures in the Winter are more limiting here than a lack of moisture. *S. lycopersicoides* is frequently sympatric with *L. chilense* and *L. peruvianum*, but prefers slightly more mesic sites than the *Lycopersicon* spp.

Threats to Genetic Resources

This trip afforded an excellent opportunity to observe these wild tomatoes in their native habitat, to note any factors which might endanger the long term viability of any of them, and to record changes in populations since the previous trips made in 1986-1988. Of the 4 species we collected, *S. lycopersicoides* appears to be at most risk due to its narrow habitat requirements and limited distribution. In fact, some populations collected in the late 1980's now appear to be extinct; for instance, in the valley below Camiña, we could not find the populations that had previously been collected at Moquella, Quistagama, and Cuisama. Similarly, we were eager to collect the southernmost population of this species, observed previously by Luis Faundez around Chiapa, near Chusmisa, yet it could not be found either. In these areas, villagers typically herd goats and cattle at lower elevations, and llamas, sheep, and/or alpacas at higher altitudes. *S. lycopersicoides* is particularly vulnerable to grazing pressure due to its long fruit maturation period (6 months to a year), failure of fruit to abscise when ripe (unlike *Lycopersicon*), and low reproductive output (fruit contain only 5-10 seeds each), all of which make them particularly vulnerable to grazing pressure. In addition, the preference of this species for more mesic, high elevation sites, not only limits the amount of suitable habitat, but means that it is more often found on the 'greener' slopes where villagers herd animals. The absence of mature fruit on many plants, recently browsed shoots, and presence of herbivores nearby, all reinforce our

concern regarding the future prospects of *S. lycopersicoides*. For this reason, we recommend that some form of conservation program be initiated for this species.

In the case of *S. sitiens*, the extreme aridity of its native habitat and consequent lack of vegetation appears to make grazing of goats or other herbivores unattractive. On the other hand, mining operations do represent a potential threat, as the mines, which are common in this region, are typically of the 'open pit' type, hence involve removal of the surface material, plants and all, and also lead to more construction of roads and infrastructure (which also benefit the plant collector trying to reach remote sites).

We found *L. chilense* to be the most abundant and widely distributed species of the four we collected on this trip, and populations were generally quite large, though somewhat smaller along the coast than in the mountains. *L. peruvianum* was somewhat more limited in its distribution, as it has not been found South of the Camiña valley. The main threats to these two species appear to be intensive agriculture practiced in the major river valleys such as Azapa; however, even here they manage to grow on the edge of fields, irrigation ponds, etc. In addition, loss of habitat due to urbanization is a problem around the larger coastal cities, particularly Arica.

Participants

The people who took part in this collecting trip are listed below with their respective contact information. Each person contributed in important ways to the success of this expedition and their help is greatly appreciated. Ricardo Pertuzé was instrumental in coordinating various aspects of this project, including getting the approval of the Chilean authorities, arranging for the participation of Luis Faúndez, securing lodging for us in Santiago, and negotiating a vehicle rental agreement with Hertz; Ricardo also organized a visit to the Piga Seeds company outside of Santiago. Ricardo is currently finishing a PhD in Genetics at UC-Davis, after which he will return to his teaching position at the Universidad de Chile. Ricardo's research program is expected to emphasis plant breeding and germplasm conservation in tomato. As such, he will be a valuable contact for future collaborative projects between the TGRC and Univ. Chile. One possible project we have discussed is to carry out an ecological study of wild tomatoes in their native habitat.

Luis Faúndez is a botanist with the Univ. de Chile with extensive knowledge and field experience in the Atacama desert region. He is probably Chile's authority on the status of endangered plants in this area, and consults frequently with private companies planning mining operations, and government agencies working to conserve native vegetation. It's not surprising therefore that Luis contributed to our effort in many ways. He was our expert on local road conditions, and helped us locate several previously uncollected populations of *Lycopersicon* and *Solanum*. Thanks to Luis' broad botanical knowledge, he was able to identify nearly every plant we encountered, and our resulting catalog of native species found in association with wild tomatoes represent the most comprehensive such observations that I have know of.

Elaine Graham handled the air transportation, and was responsible for taking soil samples and herbarium specimens at collection sites. She also took samples of leaf material for DNA analysis of sympatric *L. peruvianum*, *L. chilense*, and *S. lycopersicoides* populations to look for evidence of gene flow using microsatellite markers. Elaine is doing a dissertation on diversity and crossing relations in *L. chilense*, and will try to include the recent collections in her study, if possible. Among the questions she is interested in addressing is how effectively genetic diversity has been maintained during *ex situ* regenerations at Davis.

Pedro León was, and continues to be, our contact person at INIA, and in this capacity is helped us finalize an agreement between INIA and the TGRC regarding maintenance and use of the collections. He has an interest in seed physiology during storage, and made separate seed

collections during the trip for his experiments. Pedro also helped with the logistical aspects of the trip, and has offered to have our soil samples analyzed in the lab at INIA.

Lastly, I (Roger Chetelat) was responsible for pretrip planning, arranging funding, and coordination of other participants. During the trip, I determined our daily exploration routes, objectives, and schedule. As TGRC Director, I will oversee maintenance and distribution of the accessions collected.

Collecting Agreement

Following negotiations between USDA, TGRC, and INIA, an agreement governing the conditions for maintenance and distribution of seed from these collections was worked out. This agreement is consistent with existing USDA/NPGS policy regarding germplasm distribution. While placing no restrictions on eventual use of this material, the agreement requires users to notify INIA and to at least consider returning a share of any financial gain received to the country of origin of germplasm utilized. As part of the agreement, the USDA also provided benefit sharing funds to promote the germplasm programs of the host country. These funds were used by two Chilean scientists (Gabriel Saavedra and Ricardo Pertuze) to attend a scientific conference at Davis (27-28 Sept, 2002) held to honor the contributions of Dr. Charles Rick. This also provided an opportunity for each of them to make contacts and consult with local researchers and seed company representatives.

Contact information

Roger Chetelat
Dept. of Vegetable Crops
University of California
Davis, CA 95616, USA
tel. 1-530-752-6726
fax. 1-530-752-9659
trchetelat@ucdavis.edu
<http://tgrc.ucdavis.edu>

Ricardo Pertuze
Depto. Produccion Agricola
Universidad de Chile
Casilla 1004
Santiago, CHILE
tel. 56-2- 678-5728
fax: 56-2-678-5729
rpertuze@uchile.cl

Elaine Graham
Dept. of Vegetable Crops
University of California
Davis, CA 95616, USA
tel. 1-530-754-8647
fax. 1-530-752-9659
ebgraham@ucdavis.edu

Pedro León
INIA - La Platina
Santa Rosa 11610, Parad.33 La Pintana
Santiago 439/3, CHILE
tel. 56-2-541-7223
fax. 56-2-541-6687
pleon@platina.inia.cl

Luis Faúndez
Depto. Produccion Agricola
Universidad de Chile
Santa Rosa 11315, La Pintana
Santiago, CHILE
tel. 56-2- 678-5727
fax: 56-2-678-5700
lfaundez@abello.dic.uchile.cl

II. Itinerary

The map below shows the route traveled (in red) and the location of collection sites. The collections made at each site are summarized in the table below. The following table lists the itinerary for each day of the trip, total Km, and collections numbers (#1=SAL7901, etc).

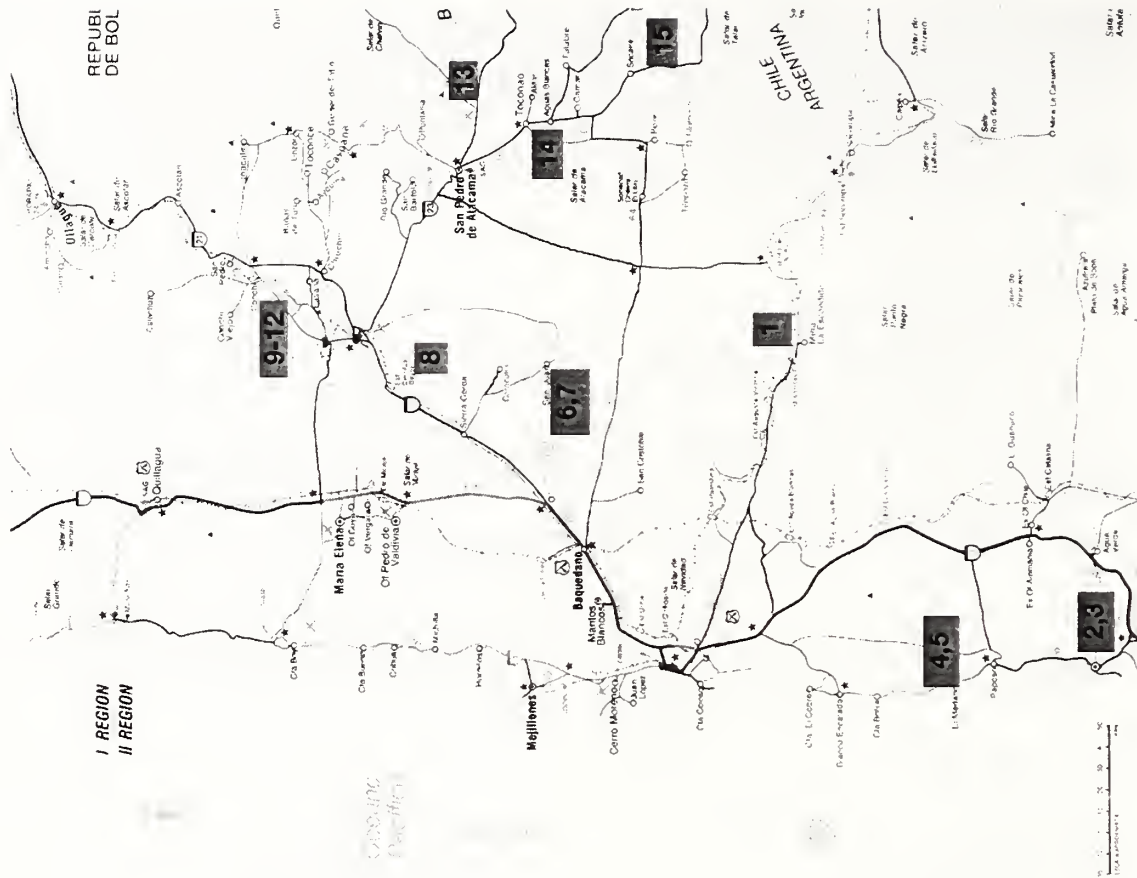
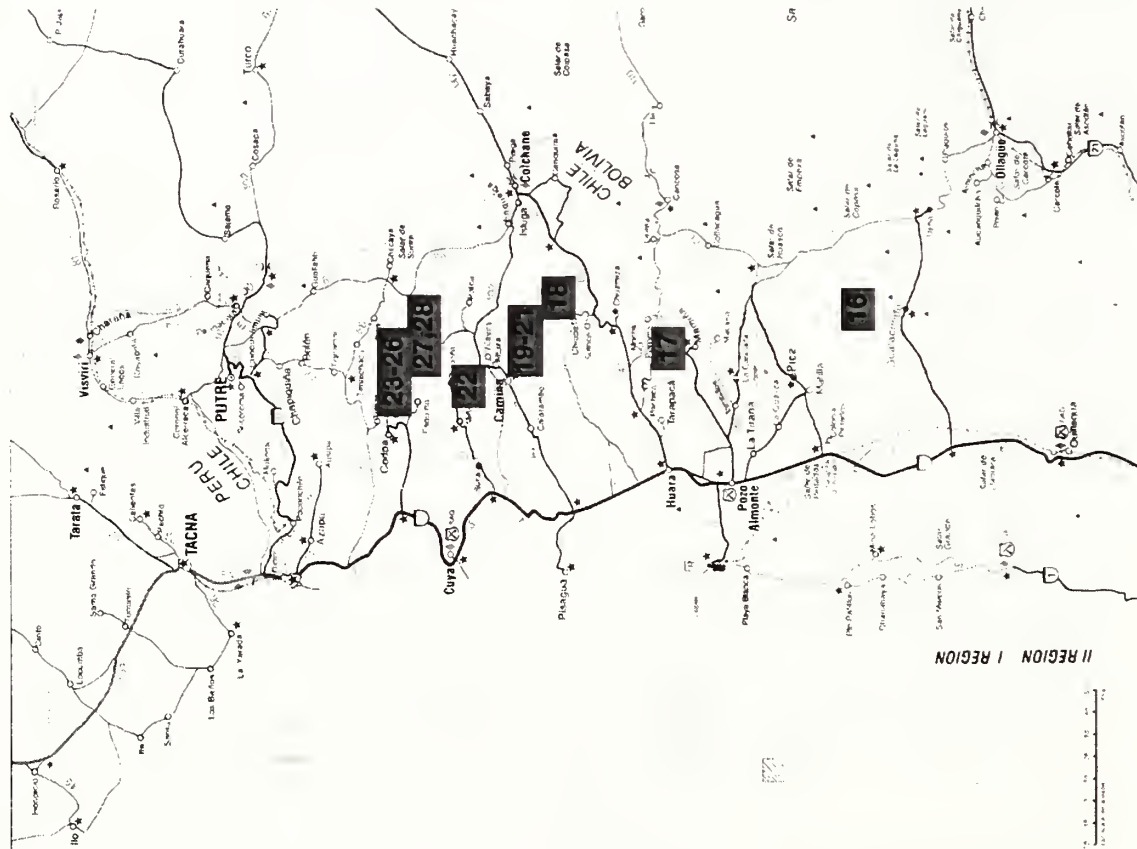


Table 1. Itinerary of collecting trip in Antofagasta and Tarapaca regions of Chile and collections made each day.

Date	Itinerary	Distance (Km)	Collection No.s
4/2	Antofagasta, Puente Coloso, Blanco Encalada, Antofagasta	313	
4/3	Antofagasta, Mina La Escondida, El Boquete, Antofagasta	396	SAL7901
4/4	Antofagasta, Taltal, Paposo, Caleta Rincon, Antofagasta	720	SAL7902, 7903, 7904, 7905
4/5	Antofagasta, Mina San Juan, Calama	310	SAL7906, 7907
4/6	Calama, Aguada Limon Verde, Cere, Quebrada Paqui, Calama	207	SAL7908, 7909, 7910, 7911, 7912
4/7	Calama, San Pedro de Atacama, Paso Jama, San Pedro de Atacama	223	SAL7913
4/8	San Pedro de Atacama, Rio Grande, Socaire, San Pedro de Atacama	350	SAL7914, 7915
4/9	San Pedro de Atacama, Calama, Cahuisa, Pica	616	SAL7916
4/10	Pica, Pachica, Guasquinao, Chusmisa	282	SAL7917
4/11	Chusmisa, Sotoca, Chiapa, Iquique	232	SAL7918
4/12	Iquique, Camiña, Nama, Camina	252	SAL7919, 7920, 7921, 7922
4/13	Camina, Pachica, Codpa, Pachica	340	SAL7923, 7924, 7925, 7926
4/14	Pachica, Esquina, Ullapata, Arica, Putre	328	SAL7927, 7928
4/15	Putre, Alcerreca, Putre	224	
4/16	Putre, Chungara, Arica	276	
4/17	Arica, Azapa valley, Arica	112	

III. Catalog of Collections

For more detailed passport information, see <http://tgrc.ucdavis.edu>.

Accession	Collection	Taxon	Collection Site	Region	Pop	Sample	Elevation (m)
LA4105	SAL7901	<i>S. sitiens</i>	Mina La Escondida	II	20+	19	2590
LA4106	SAL7902	<i>L. chilense</i>	Taltal	II	3+	2	446
LA4107	SAL7903	<i>L. chilense</i>	Catarata Taltal	II	28+	8	86
LA4108	SAL7904	<i>L. chilense</i>	Caleta Punta Grande	II	50+	8	80
LA4109	SAL7905	<i>L. chilense</i>	Quebrada Canas	II	8+	4+	75
LA4110	SAL7906	<i>S. sitiens</i>	Mina San Juan	II	14+	9	2600
LA4111	SAL7907	<i>S. sitiens</i>	Mina San Juan	II	60+	33	2700-2900
LA4112	SAL7908	<i>S. sitiens</i>	Aguada Limon Verde	II	47+	21	2800-2900
LA4113	SAL7909	<i>S. sitiens</i>	Estacion Cere	II	20+	12	2680
LA4114	SAL7910	<i>S. sitiens</i>	Pampa Carbonatera	II	35+	10	2750
LA4115	SAL7911	<i>S. sitiens</i>	Quebrada desde Cerro Oeste de Paqui	II	30+	17	2980
LA4116	SAL7912	<i>S. sitiens</i>	Quebrada de Paqui	II	100+	8	2960
LA4117	SAL7913	<i>L. chilense</i>	San Pedro - Paso Jama	II	huge	33	3540
LA4118	SAL7914	<i>L. chilense</i>	Toconao	II	11+	7	2440
LA4119	SAL7915	<i>L. chilense</i>	Socaire	II	25+	11	2980
LA4120	SAL7916	<i>L. chilense</i>	Cahuisa	I	26+	2	3170-3310
LA4121	SAL7917	<i>L. chilense</i>	Pachica - Poroma (Tarapaca)	I	65+	7	2710
LA4122	SAL7918	<i>L. chilense</i>	Chiapa	I	45+	7	3300
LA4123	SAL7919	<i>S. lycopersicoides</i>	Camina	I	50+	19	2510
LA4124	SAL7920	<i>L. chilense</i>	Camina	I	huge	0	2510
LA4125	SAL7921	<i>L. peruvianum</i>	Camina	I	50+	6	2510
LA4126	SAL7922	<i>S. lycopersicoides</i>	Camina - Nama	I	50+	11	3120
LA4127	SAL7923	<i>L. chilense</i>	Alto Umayani	I	100+	14	2370
LA4128	SAL7924	<i>L. peruvianum</i>	Pachica (Rio Camarones)	I	50	2	2530
LA4129	SAL7925	<i>L. chilense</i>	Pachica (Rio Camarones)	I	25+	2	2530
LA4130	SAL7926	<i>S. lycopersicoides</i>	Pachica (Rio Camarones)	I	20+	1	2530
LA4131	SAL7927	<i>S. lycopersicoides</i>	Esquina	I	68+	23	2170-2290
LA4132	SAL7928	<i>L. chilense</i>	Esquina	I	huge	6	2170-2290

